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# ANTIMICROBIAL ACTIVITIES OF CRUDE METHANOLIC EXTRACT AND FRACTIONS OF THE BULB OF CRINUM JAGUS (LINN)

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## ABSTRACT

*Crinum jagus* is a medicinal plant used traditionally in Nigeria to treat infectious diseases such astuberculosis andmalaria. In the present study, the antimicrobial properties of the crude extract and chromatographic fractions from the bulb of *Crinum jagus*were investigated against clinical and laboratoryisolates of bacteria and fungi using both agar well diffusion and agar dilution methods.Ampicillin (antibacterial) and tiaconazole(antifungal) were used as positive reference standard drugs.The crude plant extract and its fractionsdemonstrated broad spectrum activityagainst all the bacteriaand fungi isolatestested. Fraction 1 (24.00 mm zone of inhibition, MIC: 0.20 µg/mL, MBC:0.39µg/mL, MFC: 0.78µg/mL)demonstrated the highest activity, followed by Fraction 2 (24.00mm zone of inhibition, MIC: 0.39µg/mL, MBC: 0.78µg/mL, MFC: 1.56µg/mL. Fraction 3 (20:00mm zone of inhibition, MIC: 0.78µg/mL, MBC: 0.78µg/mL, MFC: 1.56µg/mL,). The crude extract howeverdemonstratedthe least activity against the test bacteria and fungi (18.00 mm zone of inhibition,MIC: 6.25mg/mL, MB: 25.00mg/mL, MFC: 50mg/mL.Preliminary phytochemical analysis revealed the presence of alkaloids, phenols, flavonoids,saponinsand steroids which may account for the antimicrobialactivity of theplant. The result of the study demonstrated that the extractand fractions of the bulb of *Crinum jagus* has appreciable antimicrobial properties and suggest that it may be useful in the treatment of microbial infections.

**KEYWORDS**: *Crinum jagus*, chromatographic fractions, antimicrobial activities, inhibition.

## INTRODUCTION

Worldwide, infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries (WHO, 2013). Even though a number of new antibiotics have been produced bypharmaceutical industriesin the last three decades, resistance to these drugs by microorganisms has increased and has now become a global concern(Monroe and Polk, 2000).Bacterial and fungal pathogens have evolved numerous defense mechanisms against antimicrobial agents, and resistance to old and newly produced drugs is on the increase. The increasing failures of chemotherapeutic agentsand antibiotic resistance exhibited by pathogens have led to the continuous search and screening of several medicinal plants for potential antimicrobial activity. The use of plant-derived compounds to treat microbial infection is an agelong practice in many parts of the world, especially in developing countries where there is dependence on traditional medicine for variety of diseases (Shiba*et al*., 2005;Gangoue *et al*., 2006). A vast number of medicinal plants have been recognized as valuable sources of natural antimicrobial compounds (Maliady, 2005), although their efficiency and mechanisms of action have not been tested scientifically in most cases. Plant-derived products contain a great diversity of phytochemicals such as phenolic acids, flavonoids, tannins lignin and other small compounds (Cowan, 1999).Thesecompounds possess numerous pharmacological effects such as antibacterial, antifungal, anticarcinogenic and vasodilatory activities (Bidlack*et al.*, 2000).*Crinum jagus*(Linn)is a bulbous plant with umbels of lily-like flowers and spirally arranged leaves found in tropical and substropical regions of the world (Mabbery, 1991). It belongs to the family Amaryllidacea, Phylum-Angiospemae and Subphylum-Lilifloral. The local names of the plant are OgedeOdoin Yoruba, Alubarhain Edo and Oyimbakar in Efik/Ibibio. The plant may be found in swampy conditions, seasonal wetlands or in grasslands (Savannah). The plant attracts attention due to various medicinal properties such as antibacterial and antifungal activities (Adesanya*et al.*,1992), anticholinergic activity (Peter *et al*., 2004), anti-snake venom activity (Ode and Asuzu, 2006), antioxidant and anti-haemorrhagicactivities (Ode *et al.*, 2010), antitubercular activity (Idu*et al.*, 2010) and anticonvulsant activity (Azikwe and Siminilayi, 2012).Hence in the present study, the antibacterial and antifungal activities of crude methanolic extract and fractions of the bulb of *Crinum jagus* against several human pathogenic bacteria and fungi were investigated to provide scientific evidence for the traditional use of the plant for the treatment of suspected microbial infections and identification of its active principles.

## MATERIALS AND METHODS

### Plant Material

The bulb of *Crinum jagus*were collected from Omi-Adio area, a surbubin Ibadan, Oyo State and were authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen of the plant (FHI -10911) was deposited in the herbarium of the Institute.

### Microorganisms

The microorganisms used in this study were clinical isolates obtained from the Department of Pharmaceutical Microbiology, University of Ibadan. The organisms were maintained on agar slant at 4°C and subcultured on a fresh appropriate agar slant24 hours prior to the antimicrobial test. Two Gram-positive bacteria :*Staphylococcus aureus, Bacillussubtilis*; four Gram-negative bacteria. *Escherichia coli, Pseudomonas aeruginosa, Salmonellatyphi, Klebsiellapneumoniae* and six fungi :*Candidaalbicans, Candida tropicalis, Candidakrusei, Penicilliumnotatum, Aspergillusniger* and *Aspergillusflavus* were used for the bioassay.

### Preparation of the Crude Extract

Fresh samples of the plant materialwere rinsed to remove dirts and then wiped dry. They were chopped, air-dried and ground into powdery form. About 1,127 grammes of thepowdered plant was loaded into soxhlet extractor and then defatted with boiling petroleum ether, followed by methanol for 24hours. The supernatant containing petroleum ether and methanol were allowed to evaporate using water bath. A brown viscous semi-solid substance was obtained and transferred into a clean dry bottle, weighed and labelled. This was stored in the deep freezer prior to use.

### Preparation of the Fractions

The crude methanolic extract of the plant was fractionated by column and preparative thin layer chromatography. A glass column was packed with silica gel (60-200 flash chromatography grade) using *n*-hexane under positive pressure. The crude extract was adsorbed with the silicagel, dried and packed into the column layer. Three solvents (hexane, ethyl acetate and methanol were used in order of their increasing polarity)to elute the column. Twenty one fractions were obtained. The fractions were pooled together following thin layer chromatography(TLC) fingerprinting. Thin layer chromatography was carried out using analytical silica gel pre-coated plates. The fractions were spotted on TLC plates using capillary tubes. The spotted plates were developed in a chamber saturated with ethylacetate/methanol (9:1) as mobile phase. Spots which gave purple under ultraviolet light (354nm) and possessing similar Rf values (retardation factor) were used as a criteria for deciding fractions to be pooled together. This reduced the number of the fractions to five. The biological activities of three fractions(F1, F2 and F3)out of the five fractions were tested because of the insignifi-

cant yield for the other two fractions obtained from the *n*-hexane elution.

**Preliminary Phytochemical Screening**

The extract and its fractions were subjected to standard phytochemical analysis for different constituents such as alkaloids, flavonoids, saponins, phenols, tannins and steroids as described by Edeoga*et al*.,(2005)

**Antimicrobial Assays**

The antimicrobial screening was carried out using the agar well diffusion method as described by Linoand Deogracious, (2006). The bacterial cultures were inoculated in nutrient broth (Oxoid) and incubated for24 hours at 37°C while the fungal cultures were inoculated on potato dextrose agar (Oxoid) andincubated for 48hours at 28°C.Adequateamount of Mueller Hinton agar (Oxoid) were dispensed into sterile plates and allowed to solidify under as epticconditions. Each of the culture was then adjusted to 0.5 McFarlandturbidity standard and innoculated (0.2mL each) onto Mueller Hintonagar plates. A sterile cork borer was then used to make wells (6mmdiameter) for different concentrations of the extract and each of the fractions on each of the culture of different test organisms. The extract and the fractions were separately redissolved in methanolat concentrations of 6.25, 12.5, 25, 50,100 and 200mg/mL for the crude extract and 0.78, 1.56, 3.125, 6.25, 12.5 and 25µg/mLforeach of the fractions and 0.5mL of different concentrations of the extract and each of the fractions were then introduced into the wells using sterile Pasteur pipettes. A 0.5mL portionof sterile methanol was introduced into another well to serve as negative control.Wells containing standard antimicrobials :ampicillin (10µg/mL) and tiaconazole (10%w/v) were included as positive controls. The bacterial plates were incubated at 37°C for 24hours,while the fungal plates were incubated at28°Cfor 48hours. After incubation,all plates were observedfor zones of growth inhibition and the diameter of these zones were measured in millimeters. All tests were performed under sterile conditions in duplicate and repeated three times.

**Determination of Minimum Inhibitory Concentration (MIC),Minimum Bactericidal Concentration(MBC)andMinimum Fungicidal Concentration(MFC)**

Determination of the minimum inhibitory concentration (MIC) was carried out using the broth dilution method (Sahmand Washington, 1990;Oyeleke*et al*., 2005). For the bacterial isolates, six different concentrations of the crude extractranging from 1.56 -50mg/mLwere prepared while five different concentrations of each of the fractions ranging from 0.95 - 3.12µg/mL were prepared. To determine the MIC of the fungal isolates, six different concentrations of the extract ranging from 6.25 – 200mg/mLwere prepared while different concentrations of each of the fraction ranging from 0.195– 3.125µg/mLwere also prepared. A 2mLportion of each of the different concentrations of the extract and fractions was added to 18mLof agar in test tubes making up the volume to 20mL.Then 1mLof an18hours old of each of the bacterial and fungal cultures earlier adjusted at $10^8$CFU/mLwas added to each test tube. For bacteria cultures, the tubes were incubated at 37°Cfor24hours, while for the fungal cultures, the tubes were incubated at 28°Cfor 48hours and observed for growth in form of turbidity. The lowest concentration of the extract and the fractions that produced no visible bacterial or fungal growth (turbidity) by visual inspection was considered the MIC. The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by removing 100µLof bacterial and fungal suspension from the MIC tubes that did not show any growth and subcultured on to Mueller Hintonagar plates and incubated at 37°Cfor 24hours for bacterial cultures and 28°Cfor 48hours for fungal cultures. After incubation, the concentration at which no visible growth was seen was recorded as the MBC or MFC.

**RESULTS**

Phytochemical screening revealed the presence of alkaloids, flavonoids, saponinsphenols, tannins and steroids.The antibacterial and antifungal activities of the extract andfractions of the bulb of *Crinum jagus* examined in this studywere qualitatively and quantitatively assessed in terms of inhibition zones (Tables1, 2, 3, and 4),MIC, MBC and MFC (Table 5). The crude extract exhibited considerable level of inhibition against all the test organisms even at low concentrations with exception of *E.coli*. *Klebsiellapneumoniae* was the most susceptible bacterium to the extract with inhibition zone rangingfrom 10.00 ± 0.20 to 28.00 ± 0.10, while *Aspergillusniger* was the most susceptible fungi with inhibition zone ranging from 10.00 ± 0.10 to 16.00± 0.40. The result of the MIC,MBC and MFC of the extracts are presented in Table5. The MIC of the crude extract against the tested bacteria ranged between 3.125 - 50mg/mL while the MIC for fungal isolates ranged between 25 – 200mg/mL. The broadest bactericidal activity of the extract against most of the test bacterial was 25mg/mLas MBC. *Candidaalbicans* and *Candida tropicalis* were more susceptible to the extract with 50mg/ml as MFC. All the three fractions (F1, F2 and F3) inhibited the growth of all the test organisms including *E.coli* which was not inhibited by the crude extract (Tables 2, 3, and 4). The results for the determination of MIC, MBC and MFC showed that F1 was the most potent of all the three fractions. The MIC, MBC, and MFC value ofF1for bacterial and fungal isolated were much lower than that of F2 and F3. The MIC value of F1 ranged between 0.20 to 3.125µg/mLfor bacterial isolate and 0.39 to 3.125µg/mLfor fungal isolates. *Staphylococcus aureus* was the most susceptible bacteria to F1 with MIC value of 0.20µg/mLwhile the yeasts ;*Candida albicans, Candida tropicalis* and *Candida krusei* were much sensitive to F1with MIC value of 0.39µg/mL. *E.coli* which was not inhibited by the crude extract of the plant was inhibited by F1 at MIC value of 0.39µg/mL. The MBC (0.39µg/mL) and MFC (0.78µg/mL)value of F1 for bacteria and fungi were much lower than that of F2 (Table 5). Fraction 2 had a moderate antibacterial and antifungal activity against the test organisms. The lowest MIC value ofF2 was 0.39µg/mLagainst two of the bacteria isolates:*Staphylococcus aureus* and *Bacillus substilis* and the lowest MIC value of 0.78µg/mLwere observed for fungal isolates:*Candida albicans, Candidatropicalis, Candida krusei* and *Penicillumnotatum.* Also the MBC (0.78µg/mL) and MFC (1.56µg/mL) values ofF2 for bacterial and fungal isolates were higher than that of F1 (Table 5). F3 demonstrated the leastantimicrobial activity of the three fractions. The lowest MIC value obtained for F3 was 0.39µg/mL against *Bacillus substilis* and *E.coli* and the lowest value of 1.56µg/mLwas observed for thefungal isolates which was higher than MIC value of F1 and F2. The MBC and MFC value of F3 for bacterial and fungal isolates were also higher than that of F1 and F2 hence F3 will have significant antimicrobial activity against bacterial and fungal at higher concentrations. Ampicillin and tiaconazole demonstrated the highest activities against both bacteria and fungi respectively. Result of study showed that the crude extract and fractions of the bulb of *Crinum jagus* demonstrated a broad spectrum of antimicrobial activity against Gram-positive and Gram-negativebacterialand fungal srainstested.

**Table 1: Antimicrobial activity of the crude methanol extract of the bulb of Crinum *jagus*.**

| Conc | S. *aureus* | B. *Subtilis* | E. *coli* | P. *aeuriginosa* | S. *typhi* | K. *pneumonae* | C. albicans | C. *tropicalis* | C. *krusei* | A. niger | A. flavus | P. notatum |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 200mg/ml | 18.00 ± 0.32 | 18.00 ± 0.01 | - | 18.00 ± 0.02 | 18.00 ± 0.20 | 18.00 ± 0.10 | 14.00 ± 0.30 | 12.00 ± 0.30 | 12.00 ± 0.10 | 16.00 ± 0.40 | 14.00 ± 0.30 | 12.00 ± 0.01 |
| 100mg/ml | 16.00 ± 0.20 | 20.00 ± 0.20 | - | 12.00 ± 0.40 | 16.00 ± 0.10 | 18.00 ± 0.41 | 10.00 ± 0.40 | 10.00 ± 0.20 | 10.00 ± 0.20 | 12.0 ± 0.20 | 12.00 ± 0.01 | 10.00 ± 0.10 |
| 50mg/ml | 14.00 ± 0.10 | 18.00 ± 0.06 | - | 10.00 ± 0.42 | 14.00 ± 0.10 | 16.00 ± 0.60 | - | - | - | 10.00 ± 0.10 | 10.00 ± 0.10 | - |
| 25mg/ml | 12.00 ± 0.01 | 16.00 ± 0.10 | - | - | 12.00 ± 0.30 | 14.00 ± 0.30 | - | - | - | - | - | - |
| 12.5mg/ml | 10.00 ± 0.12 | 14.00 ± 0.20 | - | - | - | 12.00 0.22 | - | - | - | - | - | - |
| 6.25mg/ml | 6.00 ± 0.01 | 0.80 ± 0.02 | - | - | - | 10.00 0.20 | - | - | - | - | - | - |
| Ampicillin (10g/mL) | 50.00 ± 0.42 | 28.00 ± 0.10 | 36.00 ± 0.10 | 34.00 ± 0.30 | 24.00 ± 0.20 | 36.00 ± 0.20 | - | - | - | - | - | - |
| Tiaconazole (10% w/v) | - | - | - | - | - | - | 24.00 ± 0.10 | 20.00 ± 0.10 | 22.00 ± 0.10 | 24.00 ± 0.10 | 20.00 ± 0.10 | 22.00 ± 0.20 |
| Methanol | - | - | - | - | - | - | - | - | - | - | - | - |

Values represent diameter of zone of inhibition (mm)
- means no inhibition

**Table 2: Antimicrobial activity of Fraction 1 (F1)of the bulb of *Crinum jagus***

| Conc | S. *aureus* | B. *subtilis* | E. *coli* | P. *aeuruginosa* | S. *typhi* | K. *pneumoniae* | C. *albicans* | C. *tropicalis* | C. *krusei* | A. *niger* | A. *flavus* | P. *notatum* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25g/mL | 20.00 ± 0.42 | 24.00 ± 0.32 | 18.00 ± 0.01 | 20.00 ± 0.02 | 24.00 ± 0.30 | 18.00 ± 0.36 | 18.00 ± 0.36 | 16.00 ± 0.31 | 16.00 ± 0.10 | 16.00 ± 0.20 | 20.00 ± 0.31 | 14.00 ± 0.30 |
| 12.5g/mL | 18.00 ± 0.25 | 18.00 ± 0.25 | 14.00 ± 0.20 | 12.00 ± 0.40 | 18.00 ± 0.20 | 16.00 ± 0.21 | 16.00 ± 0.43 | 14.00 ± 0.20 | 14.00 ± 0.30 | 12.00 ± 0.20 | 14.00 ± 0.20 | 12.00 ± 0.10 |
| 6.25g/mL | 16.00 ± 0.20 | 12.00 ± 0.10 | 12.00 ± 0.12 | 10.00 ± 0.42 | 16.00 ± 0.40 | 14.00 ± 0.30 | 14.00 ± 0.26 | 12.00 ± 0.10 | 12.00 ± 0.20 | 10.00 ± 0.10 | 12.00 ± 0.49 | 10.00 ± 0.42 |
| 3.125g/mL | 14.00 ± 0.12 | 12.00 ± 0.41 | 10.00 ± 0.02 | - | 12.00 ± 0.10 | 12.00 ± 0.15 | 12.00 ± 0.31 | 10.00 ± 0.35 | 10.00 ± 0.24 | - | 10.00 ± 0.01 | - |
| 1.56g/mL | 12.00 ± 0.11 | 10.00 ± 0.20 | - | - | - | - | 10.00 ± 0.01 | - | - | - | - | - |
| 0.78g/mL | 10.00 ± 0.50 | - | - | - | - | - | - | - | - | - | - | - |
| Ampicillin (10g/mL) | 28.00 ± 0.10 | 26.00 ± 0.10 | 28.00 ± 0.10 | 24.00 ± 0.20 | 30.00 ± 0.20 | 22.00 ± 0.25 | - | - | - | - | - | - |
| Tiaconazole (10% w/v) | - | - | - | - | - | - | 26.00 ± 0.10 | 24.00 ± 0.10 | 26.00 ± 0.20 | 24.00 ± 0.20 | 24.00 ± 0.15 | 26.00 ± 0.30 |
| Methanol | - | - | - | - | - | - | - | - | - | - | - | - |

Values representdiameter of zone of inhibition (mm)
- means no inhibition

**Table 3: Antimicrobial activity of Fraction 2 (F2)of the bulb of Crinum jagus**

| Conc | S. aureus | B. subtilis | E. coli | P. aeuruginosa | S. typhi | K. pneumoniae | C. albicans | C. tropicalis | C. krusei | A. niger | A. flavus | P. notatum |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25g/mL | 24.00 ± 0.02 | 24.00 ± 0.40 | 16.00 ± 0.24 | 16.00 ± 0.40 | 16.00 ± 0.32 | 22.00 ± 0.36 | 18.00 ± 0.41 | 16.00 ± 0.20 | 18.00 ± 0.32 | 18.00 ± 0.30 | 16.00 ± 0.50 | 18.00 ± 0.20 |
| 12.5g/mL | 18.00 ± 0.10 | 18.00 ± 0.18 | 12.00 ± 0.30 | 14.00 ± 0.50 | 12.00 ± 0.20 | 16.00 ± 0.31 | 14.00 ± 0.10 | 12.00 ± 0.10 | 14.00 0.20 | 10.00 0.10 | 12.00 0.44 | 14.00 ± 0.13 |
| 6.25g/mL | 14.00 ± 0.25 | 14.00 ± 0.15 | 10.00 ± 0.18 | 12.00 ± 0.30 | 10.00± 0.10 | 14.00 ± 0.24 | 12.00 ± 0.26 | 12.00 ± 0.10 | 12.00 ± 0.40 | - | 10.00 ± 0.35 | 10.00 ± 0.25 |
| 3.125g/mL | 12.00 ± 0.20 | 12.00 ± 0.20 | - | 10.00 ± 0.10 | - | 12.00 ± 0.15 | 10.00 ± 0.36 | 10.00 ± 0.01 | 10.00 ± 0.10 | - | - | - |
| 1.56g/mL | 10.00 ± 0.10 | 10.00 ± 0.30 | - | - | - | - | - | - | - | - | - | - |
| 0.78g/mL | 10.00 ± 0.50 | - | - | - | - | - | - | - | - | - | - | - |
| Ampicillin (10g/mL) | 28.00 ± 0.10 | 26.00 ± 0.10 | 28.00 ± 0.10 | 24.00 ± 0.20 | 30.00 ± 0.20 | 22.00 ± 0.25 | - | - | - | - | - | - |
| Tiaconazole (10% w/v) | - | - | - | - | - | - | 26.00 ± 0.10 | 24.00 ± 0.10 | 26.00± 0.20 | 24.00 ± 0.20 | 24.00± 0.15 | 26.00 ± 0.30 |
| Methanol | - | - | - | - | - | - | - | - | - | - | - | - |

Values representdiameter of zone of inhibition (mm)
- means no inhibition

**Table 4: Antimicrobial activity of Fraction 3 (F3)of the bulb of *Crinum jagus***

| Conc | S. aureus | B. subtilis | E. coli | P. aeuriginosa | S. typhi | K. pneumoniae | C. albicans | C. tropicalis | C. krusei | A. niger | A. flavus | P. notatum |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25g/mL | 20.00 ± 0.02 | 18.00 ± 0.30 | 18.00 ± 0.10 | 14.00 ± 0.25 | 16.00 ± 0.32 | 18.00 ± 0.10 | 18.00 ± 0.30 | 16.00 ± 0.10 | 14.00 ± 0.30 | 16.00 ± 0.25 | 14.00 ± 0.30 | 14.00 ± 0.20 |
| 12.5g/mL | 14.00 ± 0.12 | 16.00 ± 0.20 | 16.00 ± 0.20 | 12.00 ± 0.20 | 14.00 ± 0.28 | 14.00 ± 0.15 | 14.00 ± 0.25 | 14.00 ± 0.15 | 12.00 ± 0.25 | 12.00 ± 0.20 | 14.00 ± 0.20 | 12.00 ± 0.16 |
| 6.25g/mL | 12.00 ± 0.11 | 14.00 ± 0.22 | 14.00 ± 0.25 | 10.00 ± 0.20 | 12.00 ± 0.20 | 12.00 ± 0.20 | 12.00 ± 0.10 | 12.00 ± 0.10 | 10.00 ± 0.20 | 10.00 ± 0.15 | 10.00 ± 0.20 | 10.00 ± 0.15 |
| 3.125g/mL | 10.00 ± 0.15 | 12.00 ± 0.20 | 12.00 ± 0.20 | - | 10.00 ± 0.18 | 10.00 ± 0.18 | 10.00 ± 0.24 | 10.00 ± 0.12 | 10.00 ± 0.24 | - | - | - |
| 1.56g/mL | - | 10.00 ± 0.15 | 10.0 ± 0.10 | - | - | - | - | - | - | - | - | - |
| 0.78g/mL | - | - | - | - | - | - | - | - | - | - | - | - |
| Ampicillin (10g/mL) | 28.00 ± 0.10 | 26.00 ± 0.10 | 28.00 ± 0.10 | 24.00 ± 0.20 | 30.00 ± 0.20 | 22.00 ± 0.25 | - | - | - | - | - | - |
| Tiaconazole (10%w/v) | - | - | - | - | - | - | 26.00 ± 0.10 | 24.00 ± 0.10 | 26.00 ± 0.20 | 24.00 ± 0.20 | 24.00 ± 0.15 | 26.00 ± 0.30 |
| Methanol | - | - | - | - | - | - | - | - | - | - | - | - |

Values represent diameter of zone of inhibition (mm)
- means no inhibition

**Table 5: Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of crude extract and fractions of the bulb of *Crinum jagus***

| | Crude extract | | Fraction 1(F1) | | Fraction 2 (F2) | | Fraction 3 (F3)Fraction 3 (F3) | |
|---|---|---|---|---|---|---|---|---|
| **Organisms** | **MIC (mg/mL)** | **MBC/MFC (mg/mL)** | **MIC (µg/mL)** | **MBC/MFC (µg/mL)** | **MIC (µgmL)** | **MBC/MFC (µg/mL)** | **MIC (µg/mL)** | **MBC/MFC (µg/mL)** |
| *Staphylococcus aureus* | 6.25 | 25.00 | 0.20 | 0.39 | 0.39 | 0.78 | 0.78 | 1.56 |
| *Bacillus subtilis* | 6.25 | 25.00 | 0.39 | 0.39 | 0.39 | 0.78 | 0.78 | 0.78 |
| *Escherichia coli* | - | - | 0.39 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 |
| *Pseudomonas aeurigunosa* | 50.00 | 100 | 0.39 | 0.78 | 0.78 | 0.78 | 0.78 | 1.56 |
| *Salmononellatyphi* | 12.50 | 25.00 | 0.39 | 0.78 | 0.78 | 1.56 | 0.78 | 1.56 |
| *Klebsiellapneumoniae* | 3.125 | 12.50 | 0.39 | 0.78 | 0.78 | 1.56 | 0.78 | 1.56 |
| *Candida albicans* | 25.00 | 50.00 | 0.39 | 0.78 | 0.78 | 1.56 | 1.56 | 1.56 |
| *Candida tropicalis* | 25.00 | 50.00 | 0.39 | 0.78 | 0.78 | 1.56 | 1.56 | 1.56 |
| *Candida krusei* | 50.00 | 200 | 0.39 | 0.78 | 0.78 | 1.56 | 1.56 | 1.56 |
| *Penicullumnotatum* | 50.00 | 200 | 0.78 | 1.56 | 0.78 | 1.56 | 1.56 | 1.56 |
| *Aspergullusniger* | 50.00 | 200 | 0.78 | 1.56 | 1.56 | 1.56 | 1.56 | 1.56 |
| *Aspergillusflavus* | 100 | 200 | 0.78 | 1.56 | 1.56 | 1.56 | 1.56 | 1.56 |

## DISCUSSION

The expanding bacterial and fungal resistance to antimicrobials has become a growing concern, worldwide (Gradam, 2000). Increasing bacterial resistance is prompting a resurgence in research with the antimicrobial role of herbs against resistantstrains (Hermaiswarya*et al*., 2008, Alvianioand Alvianio, 2009).A vast number of medicinal plants have been recognised as valuable resources of natural antimicrobial compounds (Maliady, 2005). Plant derived products containa great diversity of phytochemicals such as phenolicacids, flavonoids, alkaloids, saponins, tannins and other small compounds (Cowan,1999). These compounds possess numerous pharmacologicaleffects such as antibacterial, antifungal, anticarcinogenic activities.Several authors have linked the presence of these bioactive compounds to the antimicrobial properties of crude plant extracts (Sahm and Washington,1990;Adesokan*et al*., 2007; Ogbole*et al*., 2007; Owolabi*etal*., 2007; Oyeleke*et al*., 2008). With advancements of modern techniques medicinal plants research, it is now easier to identify specific constituents and assess their antimicrobial activities. We report the findings from antiinfective effect of *Crinum jagus,*a known medicinalplant widely used in Africa as an antimicrobial agent (Adesanya *et al.*, 1992).The antibacterial and antifungal activities of crude methanolicextract and fractions (F1, F2 and F3) of the bulb of *Crinum jagus* against several human pathogens were investigated. The results of the antimicrobial screening are presented in Tables 1, 2, 3, and 4. The crude extract exhibited considerable level of inhibition against all the test organisms with the exception of *E.coli.* This is in consonance with the frequently reported cases of development of multi drug resistance to many antibiotics by bacteria of which *E.coli* is the most prominent (Alonso *et al*., 2000; Sader *et al.*, 2002). However the fractions(F1, F2 and F3) inhibited the growth of all the test organisms including *E.coli*which was not inhibited by the extract. This observation therefore supports the belief that partial fractionation of crude extract can improve biological properties of natural products. It is possible that the chromatographic fractionation has removed constituents with antagonistic activities against some other useful constituents. One of the measures of assaying the effectiveness of antimicrobial agents is to determine their MIC, MBC and MFC values, which are predictive of likely therapeutic outcomes. Agents with low activity against a particular organism usually give high MIC, MBC and MFCvalues, while a highlyreactive agent gives low values. The MIC, MBC and MFC values of the extract and fractions of the plant arepresented in Table 5. The extract demonstrated considerable antimicrobial activity with its MIC against Gram-positive bacteriaranging between6.25 - 50mg/mL and that of Gram–negative bacteria between 3.125 and 50mg/mL.The MICfor the fungal isolates ranged between 25 and 200mg/mL.The extract alsoshowed bactericidal and fungicidal activities on the bacterial and fungal isolates. *Klebsiellapnemoniae* was the most susceptible bacteria to the extract with MBC value of 12.50mg/mLwhile *Candida albicans* and *Candida tropicalis* were the most susceptible fungi with MFC value of 50mg/mL. Results from Table 5also showthat F1 was the mostpotent of all the three fractions tested. The MIC, MBC and MFC values of F1 for bacterial and fungal is olates were much lower than that of F2and F3.*Staphylococcus aureus* was the most susceptible bacteria to F1 at MIC value of 0.20µg/mL, *Candida albicans, Candida tropical is* and *Candida krusei* were much sensitive to F1 with MIC value of 0.39µg/mL. Also *E. coli* which was not inhibited by the crude extract was inhibited by F1 at MIC value of 0.39µg/mL.The MBC (0.39µg/mL) and MFC (0.78µg/mL) values for F1were lower than that of F2and F3 (MBC: 0.78µg/mL, MFC: 1.56µg/mL). The lowest MIC values of F2 were0.39µg/mL against two of the bacterial is olates and 0.78µg/mLobserved against four of the fungalisolates. Alsothe MBC and MFC values of F2 for bacterial and fungalisolates were 0.78µg/mLand 1.56µg/mLrespectively which are significantly higher than that of F1. The lowest MIC values obtained for F3 was 0.39µg/mLagainst *Bacillus subtilis,* and *E.coli*while for the fungal isolates the lowest MIC value obtained was 1.56µg/mLwhich is higherthan MIC values of F1and F2. The lowest MBC (0.78µg/mL) and MFC (1.56µg/mL) values of F3 are higher than that of FI. The test organisms used in this study are associated with various forms of human infections. *Klebsiellapneumoniae* is the most important member of *Klebsiella*genus of Entero-bacteriaceae and it is an important cause of nosocomial infection (Gupta *et al*., 1993).*E.coli*causes septiceamia and can infect the gall bladder, surgical wounds, skin lesions and the lungs(Black, 1996).Infection caused by *Salmonella typhi* is a serious public health problem in developing countries (Mastroeni, 2002). The demonstration of activities against both Gram-negative and Gram-positive bacteria and fungi is a convincing indication that the plant can be a source of bio active substances with possible broad spectrum activities. The broad spectrum antimicrobial activities of the plant extract and its fractionsmay possibly be due to the identified phytochemicals in the plant such as alkaloids, flavonoids, saponins and phenols. These classes of compounds are known to have curative activity against several pathogens and therefore could suggest the use of the plant in folklore medicine(Hassan *et al.*, 2004;Usman and Osuji, 2007). This observation is in line with the findings of Adesanya *et al*., (1992) who reported that some alkaloids present in *Crinum jagus* possess antibacterial and antifungal activities.

## CONCLUSIONS

The crude methanolic extract and fractions of the bulb of *Crinum jagus*de monstrated a broad-spectrum of activity against Gram-positivebacteria and Gram-negative bacteria and the fungi which are known to be associated with different types of infections including pneumonia, urinary tract infection, wound infection, typhoid fever and mycotic infection. Fractionation of the crude extract did not lead to loss of antimicrobial activity against the same group of isolates, but rather, the fractions were found to have a better and improved antimicrobial activity with F1 demonstrating the highest activity. The study therefore provides scientific basis for the traditional use of *Crinum jagus* for the treatment of microbial infections. Bio active substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungalinfections. Isolation, identification and purification of these phytoconstituents and determination of their respective antibacterial potencies and toxicological evaluation with view to formulating novel chemotherapeutic agents should be the future direction for investigation.

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